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Relationships of a Northern MAine Population of Amelanchier (Rosaceae)

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RELATIONSHIPS OF A NORTHERN MAINE POPULATION OF *AMELANCHIER* (ROSACEAE)

by

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ABSTRACT

Amelanchier is a genus of plants that produces seeds both sexually and by apomixis (asexual seed production). Asexuality is the dominant mode of reproduction in tetraploids (which contain four sets of chromosomes) and has created uncertainty about species delimitation in this genus. A tetraploid population of *Amelanchier* at a site called Pudding Rock on the Aroostook River in northern Maine has long been hypothesized to belong to *Amelanchier gaspensis*, a member of the *Amelanchier sanguinea* species complex. Using structural features (morphology), knowledge of the number of sets of chromosomes (ploidy level), and DNA sequence data, I tested this hypothesis. Analyses of my samples plus those obtained by others falsify this hypothesis because *A. gaspensis* does not form a distinct morphological or genetic cluster that includes the Pudding Rock population and that would warrant species status. Instead, my results confirm other data supporting the conclusion that plants that have been called *A. gaspensis* are members of a massive hybrid swarm.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Commonly called shadbush or serviceberry, *Amelanchier* consists of trees and shrubs that are widespread across North America. This genus has long been a taxonomic challenge primarily due to the presence of polyploids (containing three or more sets of chromosomes; Judd et al. 2008) that reproduce both sexually and apomictically. One group of tetraploids, *Amelanchier gaspensis* (Wieg.) Fernald & Weath., was first described as a species from the Gaspé Peninsula of Quebec (Fernald and Weatherby 1931). Jones (1946) considered this species to extend west to the James Bay and Michigan, and Fernald (1950) and Haines (2011) reported it from northern Maine.

I studied a population of *Amelanchier* at a site along the Aroostook River in Ashland, Maine, called Pudding Rock. Pudding Rock *Amelanchier* has long been considered to be *A. gaspensis*. Material I collected from this population was determined by Eric Doucette (a member of my Honor's thesis committee and a PhD candidate who works on *Amelanchier* for his thesis) to be tetraploid, like numerous previously studied samples of the sanguinea complex. Diploid organisms have two sets of chromosomes, while tetraploids have four. Diploid *Amelanchier* are mostly sexual, and tetraploids reproduce almost exclusively by apomixis (Campbell et al.1985, 1987; Weber and Campbell 1989; Campbell and Wright 1996; Dibble et al. 1998; Burgess et al. 2014). Apomixis bypasses the two steps of sexuality, meiosis and fertilization. In apomixis, the egg cell develops by apomeiosis, without meiotic reduction, and therefore has the same number of chromosome sets as the mother plant. The egg develops into an embryo without fertilization, a process called parthenogenesis (Campbell et al. 1991, Koltunow and Grossniklaus 2003).

In plants generally, diploid species commonly diverge over long periods of time, millions of years in some cases. In contrast, polyploid species can arise in as few as two generations (Rieseberg and Willis 2007). As soon as a tetraploid develops, it is reproductively isolated from and will often not mate with its diploid parents due to the difference in ploidy (Coyne and Orr 2004). Amelanchier diploids form groups that are distinct morphologically, ecogeographically, and mostly genetically (Burgess 2010, Burgess et al. in prep.). Sexual polyploids, which have not been reported in *Amelanchier*, often form distinct species that may be numerous and thereby generate some complexity. The addition of apomixis to polyploidy affects diversification and leads to complexity in two primary ways. First, apomixis replicates successful genotypes into microspecies, which are morphologically uniform and minimally differentiated from one another. Microspecies are often narrowly distributed and can be numerous, occurring by the thousands within a genus. The problem is that microspecies are like species, and their recognition makes the classification of a group difficult. A second problem is that apomicts, including those in *Amelanchier*, retain a small percentage of sexuality (1-3%) in seed production and also produce pollen sexually. As a result *Amelanchier* tetraploid apomicts hybridize with diploids and other tetraploids. Polyploids resulting from these hybridizations are apomictic (Burgess et al. 2014) and often morphologically semi-cryptic, or showing close morphological similarity, compared to diploids, making it difficult to distinguish diploids from tetraploids. Semi-cryptic ploidy variation is pronounced in *Amelanchier,* in which about 55% of traditionally recognized species contain both diploids and tetraploids (Burgess et al. 2014). In general, 12–13% of plant species contain multiple ploidy levels (Wood et al. 2009).

Sexual diploid hybrids produce unreduced gametes more frequently (27%) than nonhybrids (0.56%) (Ramsey and Schemske 1998). Fusion of an unreduced gamete (a 2x egg or

sperm; “x” refers to the number of sets of chromosomes) and a reduced gamete (a 1x sperm or egg) can result in a triploid embryo (Yamauchi et al. 2004). Triploids are often sterile, but they can have an average pollen fertility rate of 30% (Coyne and Orr 2004, Ramsey and Schemske 1998), allowing production of some 1x, 2x, and 3x gametes that, when combined with a gamete of the right ploidy level, produce a tetraploid (Ramsey and Schemske 1998, Husband 2004). The triploid thus mediates the formation of a polyploid in a pathway called the triploid bridge (Yamauchi et al. 2004). The presumed importance of diploid hybrids in polyploid formation is consistent with the observation that almost all *Amelanchier* polyploids that have been studied are derived from two or three diploid ancestors.

To determine if *Amelanchier* microspecies should be considered species, one must look to species concepts. A species concept is a definition of what constitutes a species, and due to the presence of many differing species concepts, much debate revolves around them. In fact, there are over 25 different concepts, yet there is no way to scientifically determine which should be used (Coyne and Orr 2004). The most commonly used concept is the biological species concept. This concept states that species are “groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups” (Mayr 1963). This concept refers only to organisms that interbreed, meaning species are organisms that must breed sexually, and is therefore not fully applicable to *Amelanchier*.

Among the numerous species concepts that have been proposed, one that seems particularly relevant to *Amelanchier* and other asexual groups is the differential fitness species concept (DFSC) (Hausdorf 2011). Under this concept, a species is a group of organisms that shares genes that adapt the group to a particular environmental niche. The group is considered a species as long as the genes that enable adaptation to the niche are not transferred to new groups

via gene flow. If genes conferring differential fitness were transferred, they would have a negative effect on the group receiving the genes. Although niche-adapting genes cannot be transferred, other genes can undergo gene flow with no effect on species distinction. This concept applies to asexuals because it allows gene exchange with other species while allowing the asexuals to be considered a species as long as they retain genes vital for surviving in their unique environment.

I analyzed morphological and genetic data from the Pudding Rock *Amelanchier* population and compared these data to data from other plant specimens collected by members of the Campbell lab at the University of Maine. These specimens include plants designated *A. gaspensis* from Quebec and members of the *Amelanchier sanguinea* agamic complex (Burgess 2010). My objective was to determine if the Pudding Rock *Amelanchier* belongs to *Amelanchier gaspensis*.

MATERIALS AND METHODS

Plant material- With my honors' thesis advisor, Chris Campbell, I sampled *Amelanchier* from a place along the Aroostook River in Ashland called Pudding Rock and from Caribou, Maine. I cut portions of branches and dried them in a plant press during flowering on May 28, 2014 and at leaf maturity on July 10, 2014. We labeled plants with a metal tag and took GPS coordinates to facilitate relocation. Individual height and number of stems were noted. We collected several leaves to determine the ploidy level by means of a technique called flow cytometry and also collected leaves in silica to preserve them for DNA extraction. I collected morphological data from six plants obtained by Kevin Cushman, a former graduate student in the Campbell lab

studying *Amelanchier*, in 2013 and studied a specimen collected by Alison Dibble, another former graduate student in the Campbell lab, in 1990 from the Pudding Rock site (Table 1).

Table 1. Accessions for which morphological data were obtained for this thesis.

Taxon	Collection number	Ploidy Level	Country	State	Collector	Latitude	Longitude
Pudding Rock *Amelanchier*	14-004	4x	USA	ME	M.Sheltra, C.S. Campbell	46.70904	-68.31581
Amelanchier laevis x sanguinea	14-005		USA	ME	M.Sheltra, C.S. Campbell	46.70984	-68.31422
Amelanchier bartramiana	14-006	2x	USA	ME	M.Sheltra, C.S. Campbell	46.70987	-68.31428
Amelanchier laevis	14-007		USA	ME	M.Sheltra, C.S. Campbell	46.70999	-68.31417
Amelanchier sanquinea	14-008	4x	USA	ME	M.Sheltra, C.S. Campbell	46.84675	-68.00266
Amelanchier sanguinea	14-009	4x	USA	ME	M.Sheltra, C.S. Campbell	46.84678	-68.00252
Pudding Rock *Amelanchier*	3050		USA	ME	A. Dibble		
Amelanchier gaspensis	13-452	4x	Canada	QUE	K.R. Cushman	48.02182445	-65.28081117
Amelanchier gaspensis	13-463	4x	Canada	QUE	K.R. Cushman	48.79497204	-64.97067623
Amelanchier gaspensis	13-467	4x	Canada	QUE	K.R. Cushman	49.22261448	-65.59821817
Amelanchier gaspensis	13-468	4x	Canada	QUE	K.R. Cushman	49.02080826	-66.39693836
Amelanchier gaspensis	13-470	4x	Canada	QUE	K.R. Cushman	48.7613301	-67.53538689
Amelanchier gaspensis	13-471	4x	Canada	QUE	K.R. Cushman	48.63799802	-68.10384622

I sampled plant number 14-004 from the Pudding Rock site. Little sunlight reached the plant in the mornings as it grew in a crevice in the near-vertical, north-facing rock face. Pudding Rock is believed to be calcareous in composition. The remaining plants from Ashland were downriver from the rock face and more exposed to sunlight. 14-005 is a possible hybrid of *A. laevis* Wieg. and an individual like 14-004, 14-006 is *A. bartramiana* (Tausch) Roemer, and 14-007 is *A. laevis.* All collections except 14-004 were in flower during our first sampling. On

Pudding Rock, in addition to 14-004, were seven plants with flowers and, for some of these plants, also fruits, along with three vegetative plants and many seedlings. I obtained two specimens of *A. sanguinea* (Pursh) de Candolle (14-008 and 14-009) from Caribou, Maine, near the Aroostook River but well above the bank itself, on the slope of a hill. Both plants were in full sun, and noticeably more robust than plants on Pudding Rock.

Flow cytometric determination of ploidy level- Ploidy level was determined by flow cytometry performed by Eric Doucette on accessions 14-004, 14-006, 14-008, and 14-009. Kevin Cushman determined the ploidy of accessions 13-452, 13-463, 13-467, 13-468, 13-470, and 13-471 (Table 1).

Morphological data - I measured characters (Table 2) developed by Campbell and coworkers (Burgess et al., in prep.) that differentiate species of *Amelanchier*. I measured characters 1 and 2 at the time of collection. Characters were measured using either a Carl Zeiss dissecting microscope at 8X or 12X magnification equipped with an ocular micrometer or a ruler. Some characters were measured in five replicates that were averaged for analyses (Table 2). To measure characters 37–42, I rehydrated flowers in a solution of 2% aerosol OT (Ricca Chemical Company, Arlington, Texas). Data were saved as a comma-separated values (CSV) sheet.

Table 2. Morphological taxonomic characters in *Amelanchier*

	Character	**Name**	**Character type and states**	**5X**[1]
	Stems			
1	Height	SmH	continuous	
2	Number	STM#	discrete	
3	Hairiness at flowering	SmH-f	ordinal, 0-3[2]	
4	Hairiness at maturity	SmH-m	ordinal, 0-3[2]	

	Leaves at anthesis			
5	Abaxial hairiness	Lf_abH-f	ordinal, 0-3[2]	
6	Adaxial hairiness	Lf_adH-f	ordinal, 0-3[2]	
7	Color	LfC	nominal, white (densely hairy), Green, Brown, Red	
8	Development	LfD	discrete [#unfolded/folded]	
	Leaves at maturity			
9	Length	LfL	continuous	Y
10	Width	LfW	continuous	Y
11	Texture	LfTx	ordinal, Thin, Firm, Coriaceous	
12	Abaxial vs. adaxial color	Lf_ab-adC	nominal, Paler, Equal	
13	Abaxial pubescence	Lf_abH-m	ordinal, 0-3[2]	
14	Adaxial pubescence	Lf_adH-m	ordinal, 0-3[2]	
15	Apex width	Lf_apW	continuous [width at distance of 1/10 of leaf length from apex]	Y
16	Base width	Lf_bsW	continuous [width at distance of 1/10 of leaf length from base]	Y
17	Teeth/cm at apex	Th#_ap	discrete	Y
18	Tooth width	ThW	continuous	Y
19	Tooth height	ThH	continuous	Y
20	Teeth below midpoint	TH#_bs	discrete, # teeth below middle	Y
21	Petiole length	PiL	continuous	Y
	Inflorescence			
22	Length	InL	continuous	Y
23	Hairiness of lowest pedicel	PdH	ordinal, 0-3[2]	Y
24	Length of lowest pedicel	PdL-f	continuous	Y
25	Number of flowers	Fl#	discrete	Y
26	Number of inflorescence leaves[3]	Ln_Lf#	discrete	Y

	Flowers			
27	Sepal length	SeL	continuous	Y
28	Sepal width	SeW	continuous	Y
29	Sepal adaxial hairiness	Se_adH	ordinal, 0-3[2]	Y
30	Sepal orientation[4]	SeO	ordinal	Y
31	Petal length	PaL	continuous	Y
32	Petal width	PaW	continuous	Y
33	Petal adaxial, proximal hairs	Pe_ad,prH	nominal, present/absent	Y
34	Andropetaly	Andro	nominal, present/absent	
35	Stamen number	Sa#	discrete	Y
36	Anther length	AnL	continuous	Y
37	Style number	SY#	discrete	Y
38	Style length	SyL	continuous	Y
39	Style unfused length	Sy_fusedL	continuous	Y
40	Ovary hairiness	OvH	ordinal, 0-3[2]	Y
	Fruits			
41	Lowest fruiting pedicel length	PdL-m	continuous	Y
42	Fruit hairiness	FtH	ordinal, 0-3[2]	

[1]measurements replicated almost primarily 5 times, minimally 3 times

[2]0 – [no hairs]; 1 – sparsely hairy; 2 – moderately hairy, surface mostly evident; 3 – densely hairy, surface mostly obscured (following Dickinson et al. 2008)

[3]number of leaves subtending pedicels, including the lowest

[4]erect, asending, spreading, recurved from middle, reflexed from base

Accession 3050 collected from Pudding Rock (Table 1) was an important specimen for this study because, as noted above, we collected 14-004 before it flowered. We used 3050 to

represent flowering material from this population. We are confident that 3050 and 14-004 were collected from the same kind of plant (perhaps the same genotype) because Pudding Rock is a clearly distinct, small site along the river that supports just one kind of plant. This justified the combination of flowering data from 3050 with mature leaf data from 14-004, creating an accession with a complete set of measurable characters. We labelled this resulting accession 3050_14-004. This accession will be referred to as the Pudding Rock *Amelanchier*.

Molecular data- Eric Doucette extracted genomic DNA using a DNeasy Plant Mini Kit (Qiagen Inc. Valencia California, USA).We used polymerase chain reaction (PCR) to amplify the second intron of the nuclear gene *LFY2int2d* of 14-004. Sequences were cloned, and were sequenced by the University of Maine Sequencing Facility. Chloroplast DNA (cpDNA) sequences of gene rpl-16 for 14-004 were provided by Kevin Cushman.

Morphological analyses- Data collected were explored to determine relationships among plants of interest. All analyses used the software R (R Development Core Team 2005).

Eric Doucette helped me with dataset construction and analysis. I created a morphological dataset containing plants I collected and studied plus other members of the *Amelanchier sanguinea* complex, including *A. amabilis* Wieg*., A. gaspensis, A. huronensis* Wieg*., A. sanguinea*, and plants referred to as "taxonomically unspecified." In total, 67 individual plants were compared (Table 3).

Table 3. Accessions of *Amelanchier* used for morphological analyses

Accession	Taxonomic Status	Collector	Year of Collection	State/Province
08232	*A. amabilis*	M.B. Burgess	2008	NY
08226	*A. amabilis*	M.B. Burgess	2008	NY
0617	*A. amabilis*	C. Campbell, D. Werier	2006	NY
0615	*A. amabilis*	C. Campbell, D. Werier	2006	NY

0611	*A. amabilis*	C. Campbell, D. Werier	2006	NY
0607	*A. amabilis*	C. Campbell, D. Werier	2006	NY
0604	*A. amabilis*	C. Campbell, D. Werier	2006	NY
09129	*A. amabilis*	M.B. Burgess, C.S. Campbell	2009	ONT
09131	*A. amabilis*	M.B. Burgess, C.S. Campbell	2009	ONT
08270	*A. amabilis*	M.B. Burgess	2008	QUE
08263	*A. amabilis*	M.B. Burgess	2008	QUE
08264	*A. amabilis*	M.B. Burgess	2008	QUE
08265	*A. amabilis*	M.B. Burgess	2008	QUE
08266	*A. amabilis*	M.B. Burgess	2008	QUE
08267	*A. amabilis*	M.B. Burgess	2008	QUE
08262	*A. amabilis*	M.B. Burgess	2008	QUE
08261	*A. amabilis*	M.B. Burgess	2008	QUE
08260	*A. amabilis*	M.B. Burgess	2008	QUE
n10325	*A. amabilis*	M.B. Burgess, K.R. Cushman	2010	MN
q10328	*A. amabilis*	M.B. Burgess, K.R. Cushman	2010	QUE
3094	*A. gaspensis*			Gaspe
96130	*A. gaspensis*	C.S. Campbell, C.P. Campbell, W. Wright	1996	Gaspe
Spencer	*A. gaspensis*	C.S. Campbell		Gaspe
13468	*A. gaspensis*	K.R. Cushman	2013	Gaspe
13463	*A. gaspensis*	K.R. Cushman	2013	Gaspe
13467	*A. gaspensis*	K.R. Cushman	2013	Gaspe
13452	*A. gaspensis*	K.R. Cushman	2013	Gaspe
13470	*A. gaspensis*	K.R. Cushman	2013	Gaspe
13471	*A. gaspensis*	K.R. Cushman	2013	Gaspe
2962	*A. gaspensis*			ME
96104	*A. gaspensis*	C.S. Campbell, C.P. Campbell, W. Wright	1996	QUE
9691	*A. gaspensis*	C.S. Campbell, C.P. Campbell, W. Wright	1996	QUE
96132	*A. gaspensis*	C.S. Campbell, C.P. Campbell, W. Wright	1996	QUE
96126	*A. gaspensis*	C.S. Campbell, C.P. Campbell, W. Wright	1996	QUE
13457	*A. gaspensis*	K.R. Cushman	2013	QUE
10311	*A. huronensis*	M.B. Burgess, K.R. Cushman	2010	MI
09124	*A. huronensis*	M.B. Burgess, C.S. Campbell	2009	MI
08238	Taxonomically Unspecified	M.B. Burgess	2008	NY
0602	Taxonomically Unspecified	C. Campbell, D. Werier	2006	NY

08227	Taxonomically Unspecified	M.B. Burgess	2008	NY
09133	Taxonomically Unspecified	M.B. Burgess, C.S. Campbell	2009	ONT
08256	Taxonomically Unspecified	M.B. Burgess	2008	QUE
08257	Taxonomically Unspecified	M.B. Burgess	2008	QUE
08271	Taxonomically Unspecified	M.B. Burgess	2008	QUE
0937	Taxonomically Unspecified	M.B. Burgess, C. Campbell	2009	VA
frye	*A. sanguinea*	C.T. Frye	2010	MD
14008	*A. sanguinea*	C.S. Campbell & M. Sheltra	2014	ME
14009	*A. sanguinea*	C.S. Campbell & M. Sheltra	2014	ME
CEM	*A. sanguinea*			ME
9558	*A. sanguinea*	C. Campbell	1995	ME
0985	*A. sanguinea*	M.B. Burgess & C.S. Campbell	2009	MI
10341	*A. sanguinea*	M.B. Burgess & K.R. Cushman	2010	MN
10344	*A. sanguinea*	M.B. Burgess & K.R. Cushman	2010	MN
10231	*A. sanguinea*	M.B. Burgess & K.R. Cushman	2010	NC
10232	*A. sanguinea*	M.B. Burgess & K.R. Cushman	2010	NC
0619	*A. sanguinea*	C. Campbell, D. Werier	2006	NY
08228	*A. sanguinea*	M.B. Burgess	2008	NY
08230	*A. sanguinea*	M.B. Burgess	2008	NY
9513	*A. sanguinea*	C. Campbell, W. Wright	1995	NY
09132	*A. sanguinea*	M.B. Burgess, C.S. Campbell	2009	ONT
10353	*A. sanguinea*	M.B. Burgess & K.R. Cushman	2010	ONT
10364	*A. sanguinea*	M.B. Burgess & K.R. Cushman	2010	ONT
mary	*A. sanguinea*			VA
0932	*A. sanguinea*	M.B. Burgess & C.S. Campbell	2009	VA
0942	*A. sanguinea*	M.B. Burgess & C.S. Campbell	2009	VA
10365	*A. sanguinea*/ Taxonomically Unspecified	M.B. Burgess & K.R. Cushman	2010	WI
3050_14004	Taxonomically Unspecified	A. Dibble, C.S. Campbell, M. Sheltra	1990/2014	ME

I used principle component analysis (PCA), principle coordinate analysis (PCoA), and cluster analysis (CA). PCA requires quantitative data, which includes both continuous characters, such as leaf apex width, and count characters, such as the number of teeth within a centimeter of the leaf apex. PCoA uses quantitative and ordinal data, such as leaf color and hairiness traits. PCA uses two principles to interpret data. Principle 1 states that, "In general high correlation between variables is a sign of redundancy in data." Principle 2 states "The most important dynamics are the ones with the largest variance" (Mankin 2008). This means that PCA finds in the quantitative data characters that are more or less redundant (correlated) and can be grouped together. In this way the information from 15 quantitative characters (9, 10, 15-19, 21, 22, 24, 25, 27, 28, 31, and 32 in Table 2) can be compressed into a smaller number of dimensions, typically two or three. Ultimately, a graph is created that shows each plant as a point, and the points are grouped based on their presumed genetic relatedness.

Characters used in the PCoA included 3-5, 8-10, 13, 15-29, 31, 32, and 37-39 (Table 2). The process for PCoA is similar to PCA, except it is also uses ordinal data, which cannot be used in PCA.

CA groups individual plants based on the same information as PCA and PCoA, but the end visual result is a tree (or dendrogram), showing which individuals appear to be most closely related based on their proximity and the length of the branches.

Molecular analyses- The *LFY2int2d* gene of the Pudding Rock *Amelanchier* was analyzed by Eric Doucette, and I assisted in the analysis. *LFY2int2d* gene sequences of the Pudding Rock *Amelanchier* were compared to diploid *Amelanchier* bearing this gene including *A. alnifolia* (Nutt.) Nutt. var. *alnifolia*, *A. alnifolia* var. *semiintegrifolia* (Hook.) C.L. Hitchc., *A.*

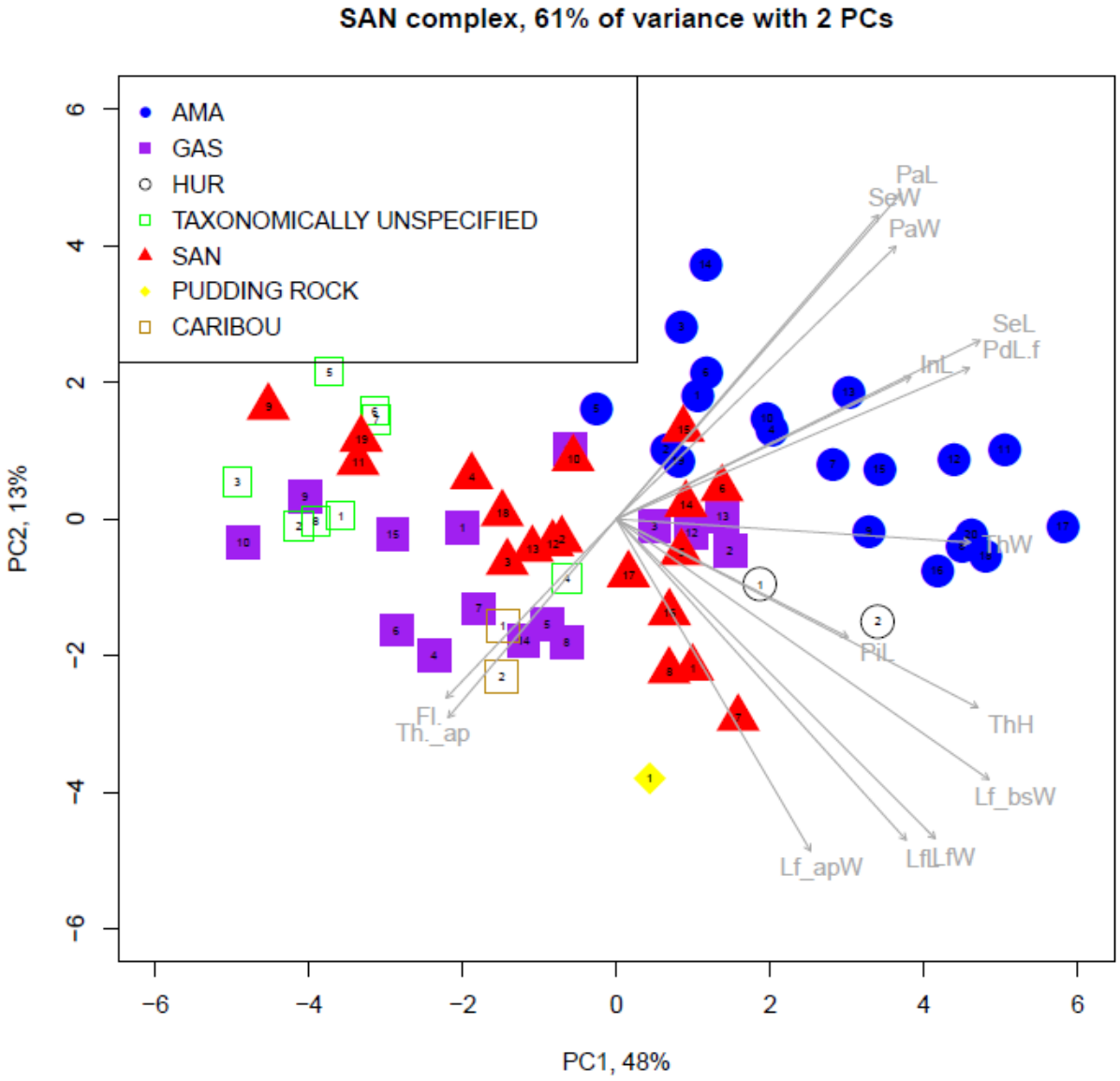
bartramiana, *A. humilis* Wieg., *A. pallida* Greene, *A. utahensis* Koehne., and *Peraphyllum* (the outgroup). Based on the DNA of these 14 accessions and nine clones of 14-004, a phylogenetic tree was created using the software PAUP* (Swofford 2001) (see Cushman et al. in prep. for an explanation of this approach) after aligning the sequences using Geneious v5.3.4. (Biomatters Ltd., Auckland, New Zealand) and SeAl v2.0a11 (Rambaut 2002).

RESULTS

Three accessions including 14-004, 14-008, 14-009 are tetraploid (Table 1;Eric Doucette, unpublished data) and accessions 13-452, 13-463, 13-467, 13-468, 13-470, and 13-471 are tetraploid (Table 1; Kevin Cushman, unpublished data).

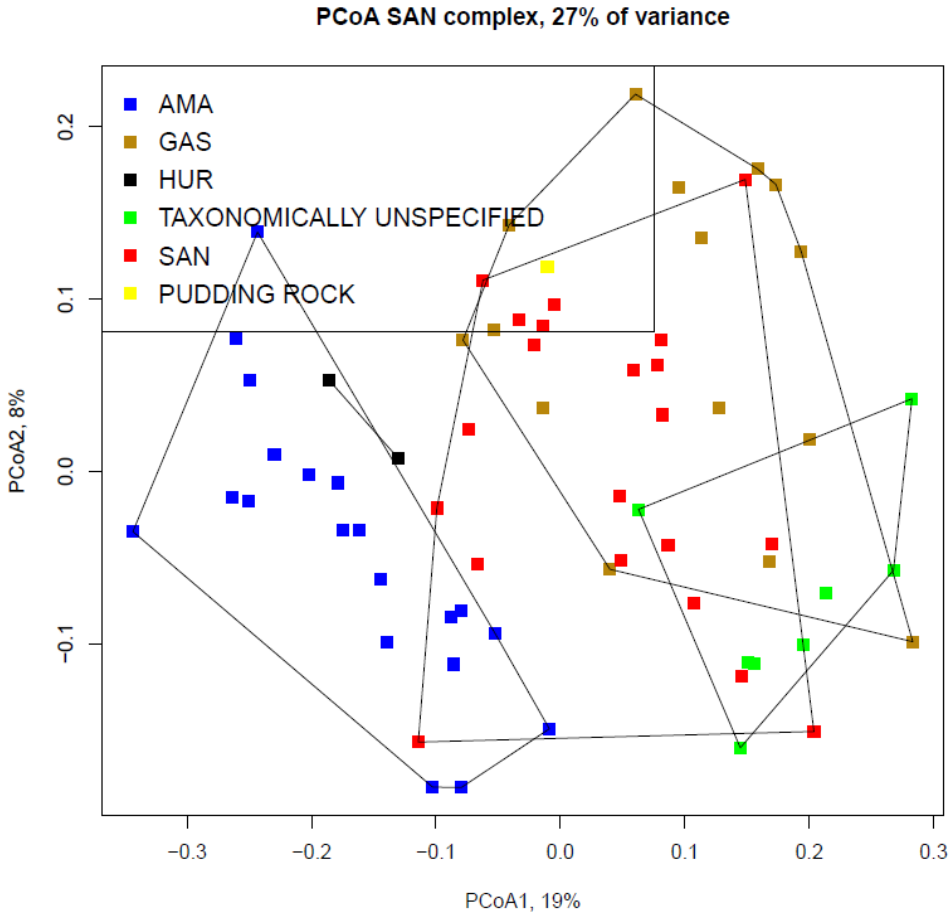
PCA (Fig. 1) showed little distinct clustering of accessions except for *A. amabilis*, which overlaps minimally with *A. sanguinea*. *Amelanchier gaspensis*, *A. sanguinea*, and the taxonomically unspecified *Amelanchier* overlapped one another extensively. Most importantly, our sample of the Pudding Rock *Amelanchier* did not fall into a well-defined cluster. Both 14-008 and 14-009 were close to the Pudding Rock *Amelanchier* in the PCA, but several accessions were more similar morphologically.

Figure 1. Principle component analysis (PCA) of Pudding Rock *Amelanchier* and *Amelanchier sanguinea* complex



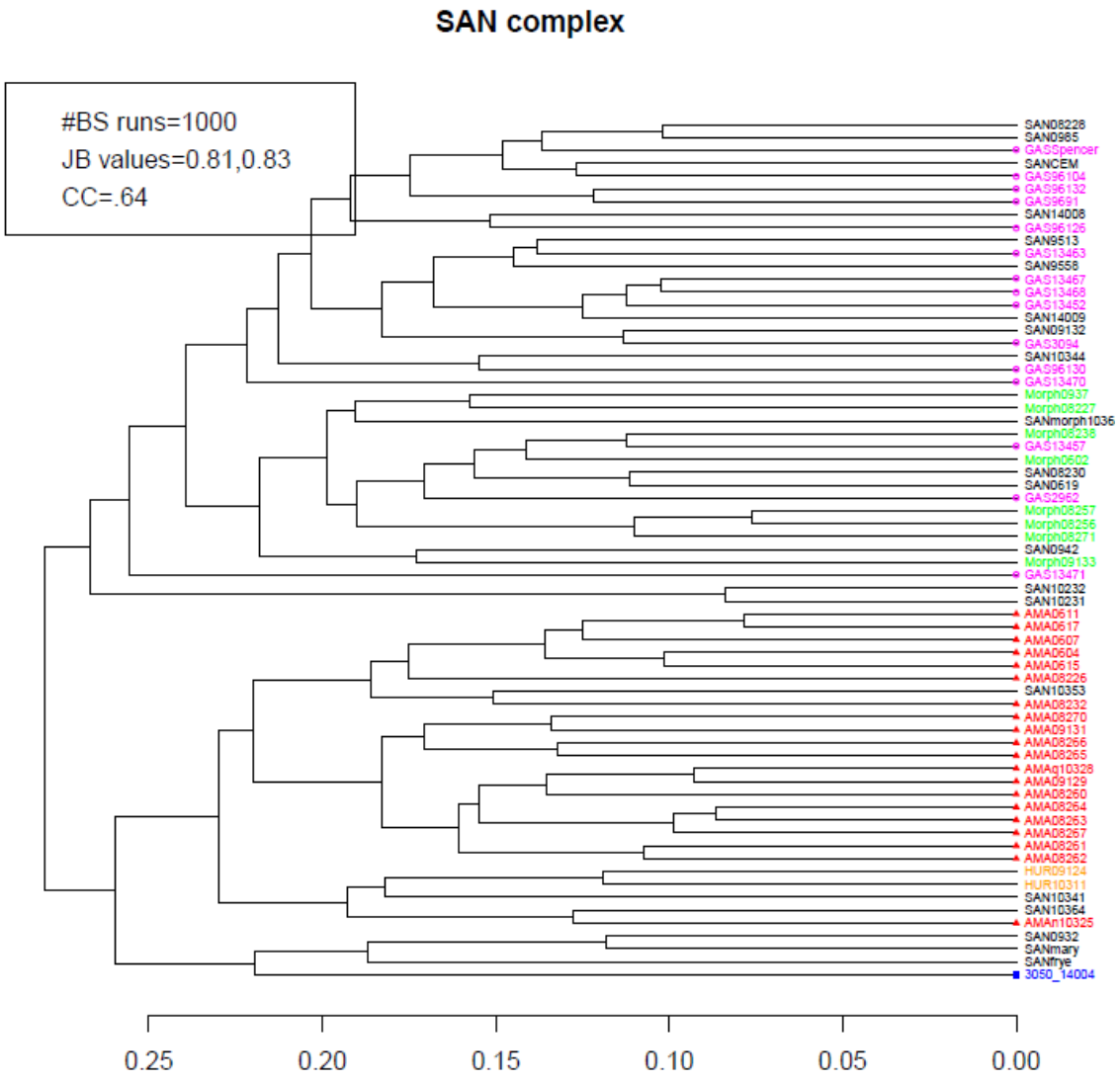
PCoA (Fig. 2) showed a pattern similar to that of PCA, with *A. amabilis* mostly distinct and no distinct clusters formed by *A. gaspensis*, *A. sanguinea*, or the taxonomically unspecified group. The Pudding Rock *Amelanchier* lies within the overlapping group of *A. gaspensis* and *A. sanguinea*.

Figure 2. Principle coordinate analysis (PCoA) of Pudding Rock *Amelanchier* and *Amelanchier sanguinea* complex



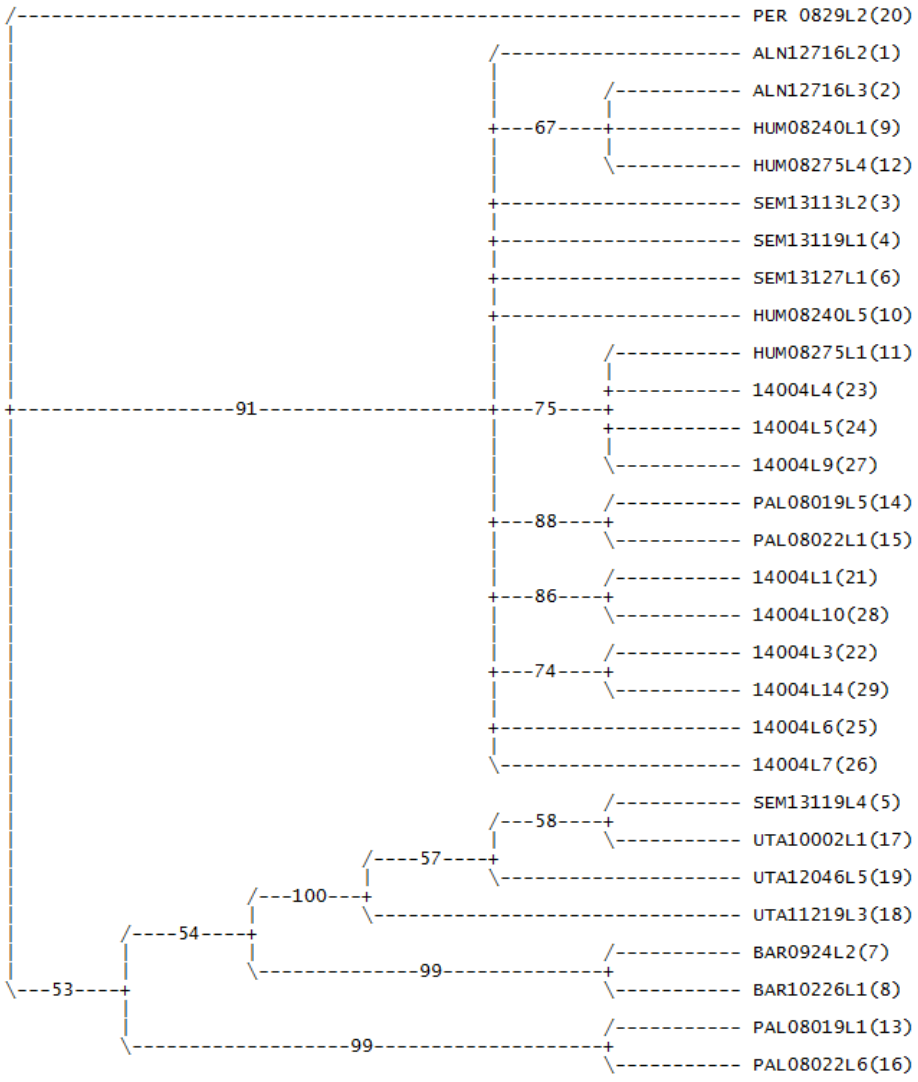
In CA (Fig. 3) the Pudding Rock *Amelanchier* is more closely related to several *A. sanguinea*, grouped with specimens of *A. huronensis*, six *A. sanguinea* specimens, and the *A. amabilis* group. The *A. gaspensis* group is intertwined with the taxonomically unspecified specimens and fifteen specimens labeled *A. sanguinea*. The Pudding Rock *Amelanchier* is in a separate cluster from 14-008 and 14-009.

Figure 3. Dendrogram of Pudding Rock *Amelanchier* (3050_14004) and *Amelanchier sanguinea* complex



The chloroplast gene rpl-16 showed that the Pudding Rock *Amelanchier* has the same mother as diploid accessions 95-129 and 06-20. In the *LFY2int2d* gene phylogenetic tree (Fig. 4), nine clones of the Pudding Rock *Amelanchier* nest with diploid *A.alnifolia* var. *alnifolia, A.alnifolia* var. *semiintegrifolia, A. humilis,* and *A. pallida.*

Figure 4. Bootstrap 50% majority-rule consensus tree based on the *LFY2int2d* gene for diploid members of the genus *Amelanchier* bearing this gene plus sequences for nine clones of accession 14-004. Sequences are represented by the first three letters of their scientific name (*A. alnifolia* var. *alnifolia*, *A. alnifolia* var. *semiintegrifolia*, *A. bartramiana*, *A. pallida*, *A. utahensis*, and *Peraphyllum* (the outgroup)), the accession number, and clone number. Sequence number is in parentheses. For further explanation, see text.



DISCUSSION

Our sample of 67 individuals of the sanguinea complex does not contain distinct subgroups apart from *A. amabilis.* While different species concepts use different bases for species status, species are defined by morphological distinctness in many species concepts

because it is assumed that morphology reflects underlying genetic distinctness that, in turn, is created during evolutionary divergence of species. Therefore, because *A. gaspensis* does not form a distinct morphological group, it should not be recognized as a species. The Pudding Rock *Amelanchier* population is not part of *A. gaspensis* because it cannot be a part of a species that does not exist. Instead, my results are consistent with the history of hybridization that has been extensively documented elsewhere in *Amelanchier*. Repeated gene flow leads to the formation of hybrid swarms, with intergradation of most groups. Groups that maintain their distinctness from this swarm are considered species of *Amelanchier.* While the proximity of the Pudding Rock *Amelanchier* to 14-008 and 14-009 in figure 1 shows morphological similarities, figure 3 places the Pudding Rock *Amelanchier* into a separate cluster. This suggests that the data are not conclusive about the relationships of these plants.

Amelanchier alnifolia var. *alnifolia, A. alnifolia* var. *semiintegrifolia*, and *A. pallida* are all from western North America (Burgess et al. in prep.), and their geographic distance from northern Maine makes them less likely contributors of genomes to the Pudding Rock *Amelanchier* than *A. humilis*, which ranges as far east as Vermont (Fernald 1950, Burgess et al. 2014). Similarly, *A. humilis* is considered to be ancestral to all other members of the sanguinea complex. Our sample of Pudding Rock *Amelanchier* is unusual in the sanguinea complex in that we have not recovered evidence of another diploid species in its ancestry. It is possible that the Pudding Rock *Amelanchier*, like almost all other *Amelanchier* polyploids that have been studied, is not of hybrid origin. It is more likely that the presence of another diploid ancestor has not been detected because we only sampled one nuclear gene from which the signal of hybridization might have been lost due to genetic recombination or segregation. The cpDNA of the Pudding Rock *Amelanchier* is very similar to that of 95-129 and 06-20. This means that they share a

mother, as chloroplasts are only passed on maternally in plants. Accession 95-129, a putative hybrid between *A.* "erecta" (a microspecies, designated as such by quotation marks) and *A. laevis,* was collected along Stillwater Avenue in Old Town. Accession 06-20 is a plant of *A. sanguinea* that was collected in western New York. The close relationship over such long distances may seem difficult to understand, but it shows that plants can disperse over considerable distances and, again, that hybridization, polyploidy, and apomixis combine to create considerable complexity.

The populations of plants located in Ashland were distinct in their habitats. The Pudding Rock *Amelanchier* and plants of the same phenotype occurred only on the rock face of Pudding Rock, while 14-005 and 14-007 occurred only downstream set back from the river's edge in sandy to gravelly soil. The Pudding Rock *Amelanchier* and similar phenotypes have not spread from the rock face to the sandy soil, and 14-005 and 14-007 do not occur on the rock face, showing distinct ecological adaptation. 14-005 is a possible hybrid between *A. sanguinea* and *A. laevis* based on morphological intermediacy of 14-005 between the Pudding Rock *Amelanchier* and 14-007 (Table 4). This putative hybridization is characteristic of extensive gene flow between different species and groups of *Amelanchier.*

Table 4. Characters demonstrating morphological intermediacy and that accession 14-005 is a hybrid between 14-004 and 14-007.

Morphological Character	14-004	14-005	14-007
Tooth number within a cm of leaf apex	4.8	6.0	8.6
Tooth width	2.65	2.26	1.53
Teeth below leaf midpoint	7.0	11.4	19.6
Petiole length	20.4	19.2	17.8
Leaf abaxial hairiness at flowering	3.0	2.0	0
Petal length	6.3	13.5	15.98

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